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Properties of single chloride selective channel from sarcoplasmic reticulum

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Abstract. The behavior of single chloride channels in sarcoplasmic reticulum of rabbit and trout skeletal muscle was examined by fusing isolated vesicle fractions into planar lipid bilayers. The channel exhibited a full open state with a unit conductance of 65 pS (in 100 m*M* Cl^-) and several subconductance states with reversal potentials which were dependent on the chloride gradient across the bilayer. Open probability was 0.6 – 0.95 for membrane potentials ranging from – 60 to + 60 mV. The kinetic behaviour could be described by assuming one time constant for the fully conducting channel, and at least two time constants for the nonconducting channel. In the presence of methane sulfonate, sulfate and phosphate anions, a decrease in the unit current amplitude but not open time argued in favor of a competition between these anions and Cl^- at the transport site of the channel. Chloride channel activity was not affected by variations of Ca^{2+} concentration in both chambers or by the presence of Mg^{2+}. Similarly, neither millimolar ATP nor the presence of the drugs taurine (up to 10 m*M*), lidocaine (2 – 40 μ*M*) or the calmodulin antagonist W7 (5 – 150 μ*M*), modified channel behavior. Finally, pH variations between 6.8 to 8 were without effect.

Key words: Anionic permeability, intracellular membrane, planar lipid bilayers

Introduction

The presence of several types of surface membrane chloride ion channels has been observed in a variety of tissues including spinal neurones (Hamill et al. 1983; Franciolini and Petris 1988), epithelial cells (Nelson et al. 1984; Welsh 1986), *Torpedo electroplax* (Tank et al. 1982; Miller and White 1984), Schwann cells (Gray et al. 1984) and skeletal (Blatz and Magleby 1985) and cardiac muscle (Coulombe and Duclohier 1984). In this paper, the bahavior of the intracellular sarcoplasmic reticulum (SR) Cl^- channel of rabbit and trout skeletal muscle was determined using the vesicle-lipid bilayer fusion technique.

There are currently available two powerful methods which allow immediate and direct observance of the behavior of a single protein molecule or complex under controllable conditions. These are the patch clamp and the vesicle-lipid bilayer fusion techniques. Solubilized channel proteins incorporated in small unilamellar vesicles (Montal et al. 1984) as well as native membrane vesicles (Miller 1978; Coronado and Latorre 1982) can be incorporated into the bilayer. The latter technique was chosen in this study for three reasons: (1) The native mammalian muscle SR membrane has been inaccessible to the patch clamp micropipette, (2) isolated sarcoplasmic reticulum vesicles are too small to perform the patch clamp technique, and (3) SR vesicles have been extensively used in the past for investigating the ionic permeabilities of this intracellular membrane (for review see Meissner 1983).

SR vesicles from both skeletal and cardiac muscle have been fused into planar lipid bilayer in order to study single channel behaviour of a K^+ channel (Miller 1978; Coronado and Miller 1982) and Ca^{2+} release channel (Smith et al. 1985, 1986a, b; Rousseau et al. 1986, 1987). Although previous ion flux measurements (Kasai et al. 1979; Yamamoto and Kasai 1981; Meissner and McKinley 1976) have reported a large anion permeability in isolated SR membrane vesicle fractions, and the presence of an anionic selective channel in the SR membrane using the bilayer technique has been reported by Miller (1978) and Suarez Isla et al. (1986) and illustrated by Smith et al. (1988),

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Abbreviations: *DIDS*: 4,4′-Diisothiocyanostilbene-2,2′-disulfonic acid; *EGTA*: Ethylene glycol *bis*-(β-aminoethyl ether) N,N,N′,N′-tetraacetic acid; *HEPES*: N-2-Hydroxyethylpiperazine-N′-2-ethane sulfonic acid; *SR*: Sarcoplasmic reticulum; *TRIS*: *Tris*(hydroxymethyl)aminomethane.

the behavior of the SR Cl^- channel has not been studied systematically on a unitary level.

In this study, we describe the functional properties of single SR chloride channels of rabbit and trout skeletal muscle under different experimental conditions. The blocking effect of different anions is demonstrated. The weak voltage sensitivity of this channel and several biochemical agents suspected of having a regulatory effect on its behavior are also investigated.

Materials and methods

Chemical reagents

Choline chloride and Trisma base (*Tris*) were obtained from Sigma Co. (St. Louis, MO). HEPES and PIPES buffers were purchased from Research Organics, Inc. (Cleveland, OH) and phospholipids from Avanti Polar Lipids (Birmingham, AL). Methane sulfonic acid, sulfuric acid and phosphoric acid, obtained from Fisher Scientific Co. (Fair Lawn, NJ), were neutralized with *Tris* to produce stock solutions at pH 7.4. All other materials were of reagent grade.

Preparation of sarcoplasmic reticulum vesicles

Vesicles were prepared by differential and sucrose gradient centrifugation as described by Meissner (1984). Light and heavy SR vesicles were taken respectively from the 30%–34% and 38%–42% regions of sucrose gradients containing membranes obtained by differential centrifugation at 35,000–130,000 × *g*. SR membrane vesicles from trout white muscle were prepared by centrifugation at 20,000 × *g* in a Sorval SS34 rotor without further sucrose gradient purification.

Buffer solutions

Since SR vesicles contain various types of ion channels which conduct mono- and divalent cations or anions, it is important that the chosen buffer does not contain permeant ions other than the one of interest. To meet this requirement, a buffer consisting a 50 m*M* choline chloride, 5 m*M* $CaCl_2$, and 10 m*M* HEPES/*Tris* (pH 7.4) was initially used in both chambers to permit formation of the planar lipid bilayer in symmetric conditions. Since choline, HEPES and *Tris* are impermeable for all practical purposes, chloride is the only significant permeable ion in solution (Meissner 1983). After the bilayer was constructed in symmetric medium, an aliquot of 1 *M* choline chloride was added in most of the experiments to the *cis* chamber to yield a final concentration of 250 m*M*. This established an osmotic gradient between the chambers which aided vesicle fusion. The presence of millimolar calcium in the fusion buffer is required to stabilize the bilayer and promote vesicle fusion. When the channel behavior was recorded under different experimental conditions, the entire content of the chambers was exchanged with the use of a perfusion pump (Havard Apparatus, Model 941) or modified by adding the compounds directly to the chambers. Glass bi-distilled deionized water was used in the preparation of all buffer solutions.

Bilayer formation and vesicle fusion

The bilayers were formed at 23 °C from a lipid mixture containing phosphatidylethanolamine, phosphatidylserine purified from bovine brain, and diphytanoylphosphatidylcholine at a ratio of 5:3:2. The final lipid concentration was 50 mg/ml dissolved in decane. A 250 μm diameter hole, drilled in a polyvinyliden-difluoride (PVDF) cup, was pretreated with the same lipid mixture dissolved in chloroform. Using a teflon stick, a drop of the lipid mixture in decane was gently spread across the hole in order to obtain formation of the bilayer membrane. Aliquots of vesicles (50 μg protein) were added to the cis chamber in the proximity of the bilayer and fusion was either spontaneous or induced by stirring or applying negative holding potentials across the bilayer. To decrease the chance of additional vesicle fusions during an experiment the free $[Ca^{2+}]$ of the *cis* chamber was lowered (< 0.5 m*M*) by addition of EGTA or by perfusion with a HEPES-*Tris* buffer solution containing 1.2 μ*M* free $[Ca^{2+}]$ (0.95 m*M* $CaCl_2$ and 1 m*M* EGTA). Note that even the fusion of a single vesicle can result in the incorporation of more than a single channel. In fact the majority of vesicle fusions resulted in multichannel recordings.

Recording instrumentation

Currents were recorded using a home-made double-stage amplifier (Smith et al. 1988) or a DAGAN 8900; then low-pass filtered (Frequency Device 902 LPF) in order to record on line channel activity on a chart recorder (Z-1000, Astro Med. Co.) and oscilloscope (Nicolet 110). The amplified currents from the bilayer were also output directly on a filter (cut off frequency 4 kHz) and recorded on a video cassette recorder (Sony SL 2700) after the signals were converted into digital form with a modified pulse code modulation device (Sony PCM 501 ES) (Bezanilla 1985). Current recordings were played back and filtered at 300 Hz and sampled at 1 pt/ms for storage on hard disk and further analysis using an IBM PC-XT computer and programs kindly provided by Dr. H. Affolter. The open probability values (P_o) and time histograms were determined from data stored in 40 s files.

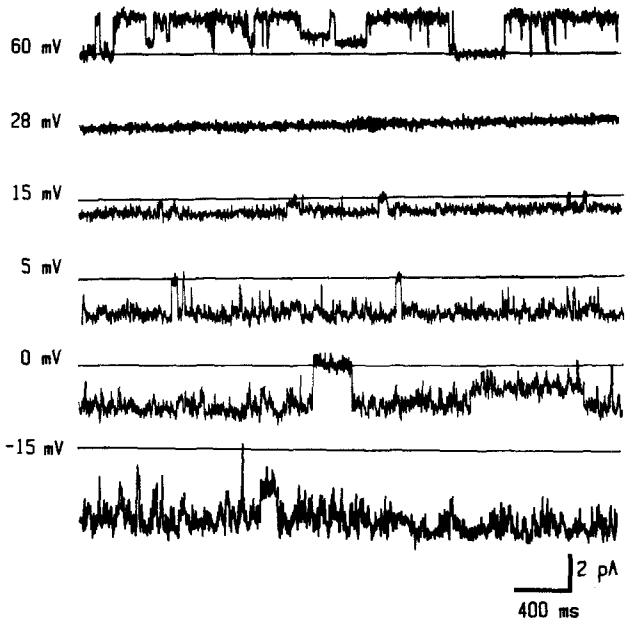


Fig. 1. Single Cl^- channel recordings at various holding potentials. A single rabbit SR Cl^- channel was recorded in the presence of 260 m*M* Cl^- *cis* and 60 m*M* Cl^- *trans*. No currents were measured at +28 mV. *Trans* to *cis* Cl^- currents are shown as upward deflections, and *cis* to *trans* as downward deflections. The *solid line* represents the zero current level

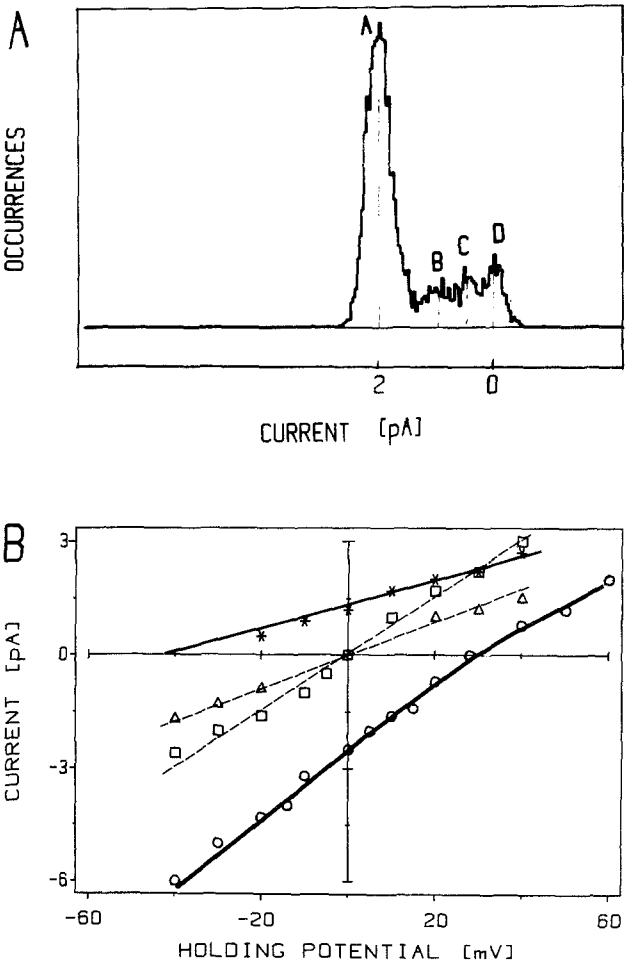


Fig. 2 A and B. Unit current amplitude analysis. **A** Current histogram plot diagram of frequency of events versus current amplitude. *A*, full conducting state; *B* and *C*, subconducting states; *D*, closed state. **B** Current voltage relationship at different Cl^- concentrations. *Circles*: 260 m*M* *cis*/60 m*M* *trans*, $\gamma = 95$ pS; *squares*: 125 m*M* *cis*/125 m*M* *trans*, $\gamma = 70$ pS; *triangles*: 60 m*M* *cis*/60 m*M* *trans*, $\gamma = 40$ pS; and *asterisks* > 2.5 m*M* *cis*/60 m*M* *trans*; $\gamma = 33$ pS. Data points represent average values from 3 to 10 experiments

Results

The initial investigation of the Cl^- channel was aimed at observing changes in channel behavior as a function of holding potential. Cl^- channel activity generally appears simultaneously with the first vesicle fusion event and is detected as an abrupt change of bilayer conductance. Figure 1 shows the current fluctuations of a single Cl^- channel recorded in asymmetric Cl^- concentration (260 m*M* Cl^- *cis*/60 m*M* Cl^- *trans*). For each applied voltage the channel displays a steady state activation for long periods of time. The upper trace has been obtained at +60 mV with open events represented as upward deflections. The amplitude of each deflection is proportional to the current passing through the channel in the form of ionic charges according to the scale shown. Another inference from this tracing concerns the presence of subconducting states. These apparent subconducting states lasted up to 0.5 s at positive holding potentials but were of shorter duration at negative voltages. The reversal potential was found at +28 mV in this experiment (second trace from the top). When the membrane is progressively hyperpolarized, a gradual increase in the unit current amplitude is observed, corresponding to an incrase in ion transport rate.

Figure 2 A shows a current histogram of a single channel recorded at +60 mV (Fig. 1, upper trace). Peak *A* represents the channel in its full open state with a mean current amplitude equal to 2 pA. Peak *D* corresponds to the zero current level of the channel in its closed state. Comparing the relative area under the peaks it is obvious that the channel is spending most of its time in the full open state. In addition, the histogram indicates the presence of intermediate current levels. The areas under *B* and *C* correspond to the presence of open states of lower conductance.

Figure 2 B shows current voltage relationship curves obtained with different Cl^- concentrations in the *cis* and *trans* chambers. In symmetric Cl^-, the unit conductance were 40 and 70 pS with 60 and 125 m*M* Cl^-, respectively. In each case, the reversal potential

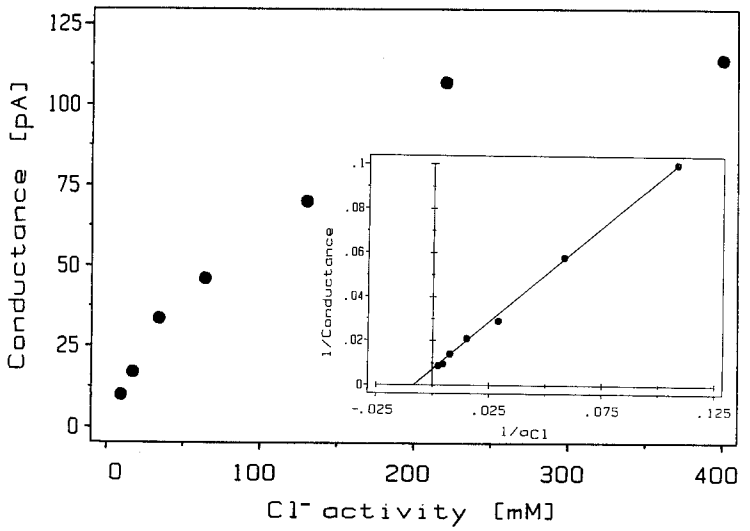


Fig. 3. Single channel conductance as a function of Cl^- activity. Data points give the average conductance of 2 to 4 experiments obtained in equal *cis* and *trans* Cl^- concentrations. Rearrangement of the data in the form of a Lineweaver-Burk plot (*inset*) indicates a maximal conductance and a K_m value of 154 pS and 125 m*M* Cl^-, respectively

was found to be 0 mV. The majority of the experiments was conducted in asymmetric Cl^- concentrations. In the presence of 260 m*M* Cl^- *cis* and 60 m*M* Cl^- *trans*, the unit conductance for the inward (*cis* and *trans*) current was 95 pS. Under the same experimental conditions but at voltages more positive than the reversal potential, the unit conductance had a lower value. The Reversal potential of +31 mV was close to the theoretical equilibrium potential value (+36.7 mV) calculated from the Nernst equation. After perfusion of the *cis* chamber with HEPES/*Tris* buffer, the Cl^- concentration was lowered to less than 2.5 m*M*. With 60 m*M* Cl^- *trans* and <2.5 m*M* Cl^- *cis* the unit conductance was found to be 33 pS and the extrapolated reversal potential was shifted toward negative values (−42 mV). The variations of the reversal potential with changing Cl^- concentrations (*cis* or *trans*) demonstrated a high Cl^- selectivity of the channel under our experimental conditions. Another way to prove the Cl^- selectivity of the channel was to perfuse both chambers with Cl^- free solution. This approach effectively eliminated Cl^- channel electrical activity, and allowed the observance of other channels such as the SR Ca^{2+} release channels (Smith et al. 1986 a, b; Rousseau et al. 1986, 1987).

Figure 3 shows single channel conductance as a function of Cl^- activity for the rabbit SR Cl^- channel. Data were recorded using symmetric Cl^- concentrations ranging from 10–500 m*M* (with the activity ranging from 9.2 to 400 m*M*). Rearrangement of the data in the form of a Lineweaver Burke plot yielded a maximum conductance (γ_{max}) of 154 pS. Cl^- conductance was half-maximal at 125 m*M*. For an intracellular Cl^- activity of 5 m*M* in skeletal muscle a unit conductance of 5 pS was calculated.

The distribution of the Cl^- channel in the SR membrane was assessed by incorporating "light" and "heavy" vesicles derived from the longitudinal and terminal cisternae regions of SR, respectively (Meissner 1984). Multichannel recordings were consistently obtained using either vesicle fraction. Accordingly Cl^- channels appear to be randomly distributed in the SR membrane. Incorporation of 3 to 5 channels per vesicle fusion in most of our experiments argues in favor of a high density of these channels.

In single channel recordings (260 m*M* Cl^- *cis*/60 m*M* Cl^- *trans*), the open probability (P_o) of the rabbit SR Cl^- channel was high (P_o = 0.60–0.90) and slightly voltage dependent. An attenuated bell-shape curve relationship with a P_o peak value near 0 mV was obtained (not shown). In symmetric Cl^- concentration (60 m*M*) the channel displayed similar high P_o values (0.45–0.8) over the same range of holding potentials (−60 to +60 mV).

SR vesicle fractions isolated from trout fish muscle contain a similar Cl^- conducting channel. Figure 4 A shows the typical bursting and flickering behavior of this channel. A high open probability and unit conductance of 65 pS and a positive reversal potential were observed in asymmetric Cl^- concentrations (260 m*M* Cl^- *cis*/60 m*M* Cl^- *trans*) (Fig. 4 B). In symmetric Cl^- concentration (60 m*M* Cl^- cis/60 m*M* Cl^- *trans*) the unit conductance of the trout channel was 58 pS, with an apparent reversal potential of 0 mV (Fig. 4 B). The unit conductance of the trout Cl^- channel at 60 m*M* Cl^- is somewhat higher than those for the rabbit skeletal (40 pS; Fig. 2) and canine cardiac SR Cl^- channels (40 pS, E. Rousseau, unpublished studies).

Time analysis of single channel fluctuations was performed in order to formulate a simplified but plausible kinetic scheme for the SR Cl^- channel. Contribution of subconductances was minimized using current traces recorded at negative voltages (Fig. 5 A). Further,

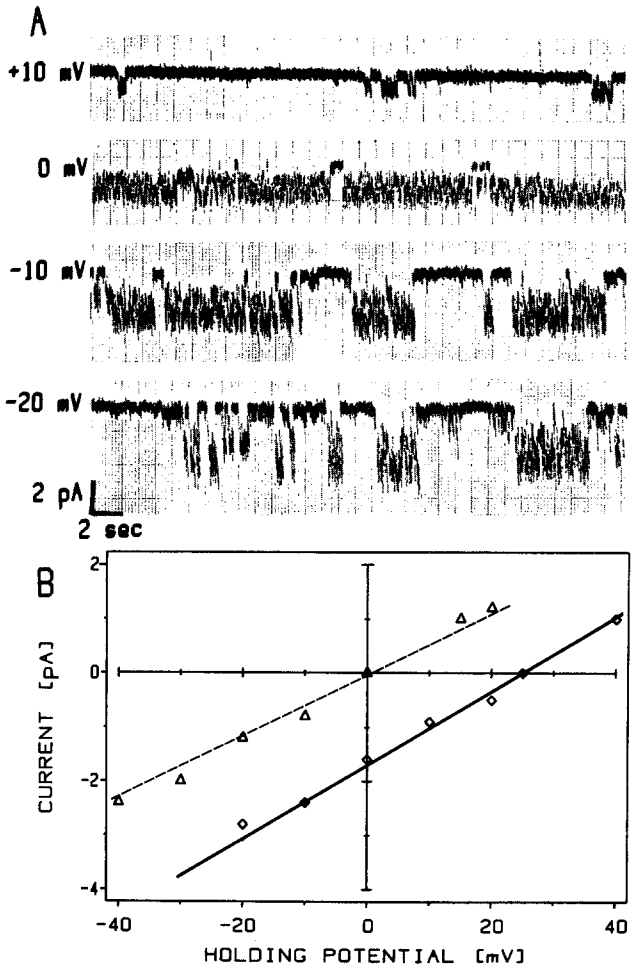


Fig. 4 A and B. Single channel activity and unit conductance of the trout SR Cl^- channel. **A** Traces recorded at different holding potentials in asymmetric Cl^- buffer (260 m*M* Cl^- *cis*/60 m*M* Cl^- *trans*). **B** Current voltage relationship for different Cl^- concentrations. *Squares:* 260 m*M* Cl^- *cis*/60 m*M* Cl^- *trans*, $\gamma =$ 65 pS; *triangle:* 60 m*M* Cl^- *cis*/60 m*M* Cl^- *trans*, $\gamma = 58$ pS

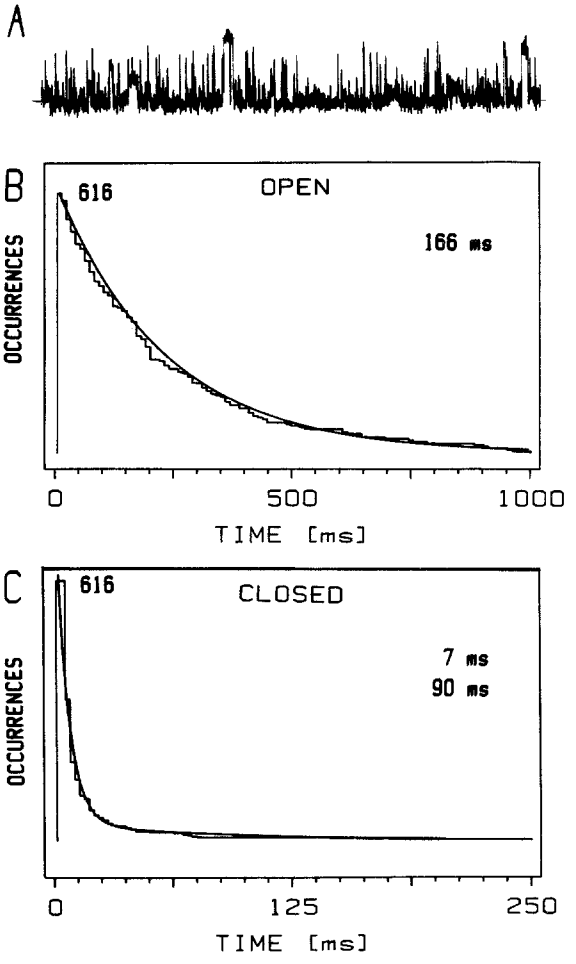


Fig. 5 A–C. Cumulative time histogram plots for open and closed states of the rabbit SR Cl^- channel. **A** Single channel recorded in 260 m*M* Cl^- *cis*/60 m*M* Cl^- *trans*, *HP* = −30 mV. **B** Open time distribution is described by one exponential with a mean open time of 166 ms. **C** Closed time distribution is described by the sum of two exponentials with mean closed times of 7 and 90 ms. Note the different time scales used for open and closed distributions

two different mathematical approaches were used (*i*) the double-threshold (Smith et al. 1986 a) and (*ii*) the half-threshold (Sachs et al. 1982) discriminator method. Although a different number of events were scored and some differences in the time constant values were obtained, both methods yielded a qualitatively similar result. Figure 5 shows two cumulative time histograms using the half-threshold discriminator method. Panel *B* represents the distribution of open events and Panel *C* that of closed events. The number of exponentials needed to describe the distribution indicates whether the distribution arose from a single population of events or a combination of a number of distinct populations (Barrett et al. 1982; Magleby and Palotta 1983). The cumulative open time histogram could be described by a single exponential (Fig. 5 B), suggesting the presence of one open state. However, at least two exponentials were needed to describe the cumulative closed time histogram, and can be correlated with the presence of two closed states (Fig. 5 C). Thus the rabbit SR Cl^- channel was found to enter at least one open state with $\tau_{op} = 166$ ms, and two distinct closed states with $\tau_{c1} = 7$ ms and $\tau_{c2} = 90$ ms. Some variations in the time constant values were observed from one experiment to another and upon changing the voltage across the bilayer. For the trout channel, one open and two closed time constants in the millisecond time range were needed to describe channel behavior within bursts. A single closed time constant in the second time range was sufficient to describe its behavior between bursts (not shown).

In Figs. 6 and 7, the behavior of the rabbit muscle SR Cl^- channel was studied in the presence of monovalent ($CH_3SO_3^-$) and divalent (SO_4^{2-}) anions which

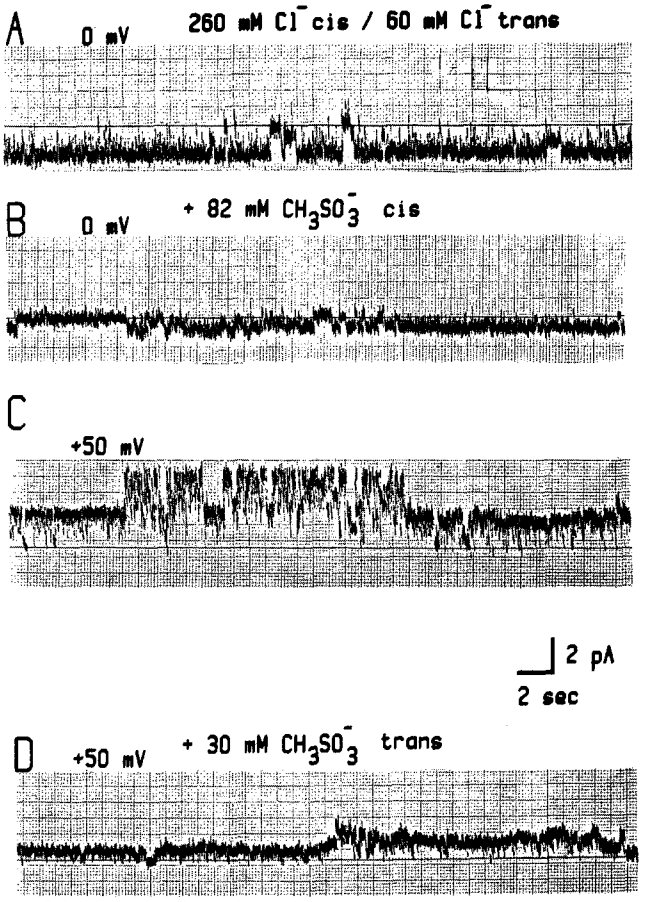


Fig. 6 A–D. Effect of methane sulfonate on Cl^- channel activity. **A** Chart recordings obtained in 260 m*M* Cl^- *cis*/60 m*M* Cl^- *trans* without methane sulfonate: $HP = 0$ mV. **B** After addition of 82 m*M* methane sulfonate to the *cis* chamber: $HP = 0$ mV. A 60% decrease of the current amplitude was observed. **C** Same conditions as in **B**, but with $HP = +50$ mV. The positive unit current amplitude was similar to the value reported on the I/V curve (Fig. 2 B) under similar experimental conditions. At this point a second current level was seen indicating activation of a second Cl^- channel. **D** After addition of 30 m*M* methane sulfonate *trans*, at +50 mV, a net reduction of the unit current amplitude was observed. The *solid line* represents the zero current level

are of larger size than the Cl^- ion. Figure 6 shows the effects of *cis* and *trans* methane sulfonate on Cl^- channel activity and unit current. The first trace (Fig. 6 A) was recorded in the presence of an asymmetric Cl^- gradient (260 m*M* Cl^- *cis*/60 m*M* Cl^- *trans*) at a holding potential of 0 mV. Figure 6 B is a recording under the same conditions except that the *cis* chamber contains in addition 82 m*M* $CH_3SO_3^-$. The channel was in the open state most of the time in both recordings. However, in the presence of methane sulfonate, the current amplitude was markedly decreased. In Fig. 6 C the conditions are the same as in B except that the holding potential (+50 mV) was more positive than the reversal potential ($HP = +30$ mV). Thus, the current was flowing in the opposite direction. The current amplitude at +50 mV was identical to the one measured in the absence of 82 m*M* $CH_3SO_3^-$ *cis* (not shown). This result suggested that $CH_3SO_3^-$ in the *cis* chamber was ineffective at decreasing the Cl^- current flowing from the *trans* to the *cis* chamber. When $CH_3SO_3^-$ was added to the *trans* chamber (Fig. 6 D) a reduction in current amplitude was again observed without modification of the open probability. The observation that there is no significant change in the time spent in the open and closed states, but a drastic decrease in current with the blocking ion on the entry side of the channel would suggest that the $CH_3SO_3^-$ anions are competing with Cl^- at a selectivity site of the chloride channel but are unable to permeate through the channel or if so, at a very low rate.

Sulfate anions affected single Cl^- channel activity in a similar manner (Fig. 7). Control recordings were obtained for two potentials (+20 and −20 mV) in order to visualize positive and negative currents in symmetric 60 m*M* Cl^- solution (Fig. 7 A). When 10 m*M* $MgSO_4$ was added to the *cis* chamber (Fig. 7 B), only a decrease in the current amplitude for negative potentials was observed without modification of the Cl^- channel activity for positive potentials. When the same amount of $MgSO_4$ was added to the *trans* chamber, only a small decrease in the current amplitude was observed for positive potentials (data not shown). Following further additions of $MgSO_4$ (to 20 m*M*) in both chambers (Fig. 7 C) a larger decrease of the unit amplitude was observed. This is particularly well illustrated in the recordings obtained at −20 mV which indicate that sulfate ions are more potent blockers from the *cis* side than from the *trans* side. For each applied voltage, comparison of the traces shows that the P_o was not significantly affected by the presence of $MgSO_4$. The reduction of the unit current amplitude (by 60% to 70%) was attributed to the presence of sulfate anion and not the divalent magnesium cation since similar effects were observed when *Tris* sulfate was used instead of $MgSO_4$. The channel's insensitivity to divalent cations was further studied by varying the free $[Ca^{2+}]$ in both chambers. Changes in free $[Ca^{2+}]$ (from 5 m*M* down to 0.1 μM) did not appreciably affect Cl^- channel conducting and gating behavior.

The effects of inorganic phosphate anions were assessed by addition of 2 to 20 m*M* *Tris* phosphate to both chambers containing 260 m*M* Cl^- *cis*/60 Cl^- *trans*. At concentrations $\leqq 5$ m*M*, P_i had no detectable effect under our experimental conditions. At higher concentrations (up to 20 m*M* in both chambers) the unit current amplitude decreased by 20%. In these experiments, the concentration of free Ca^{2+} was lowered to 100 μM by addition of EGTA to both chambers, to prevent formation of calcium-phosphate precipitates.

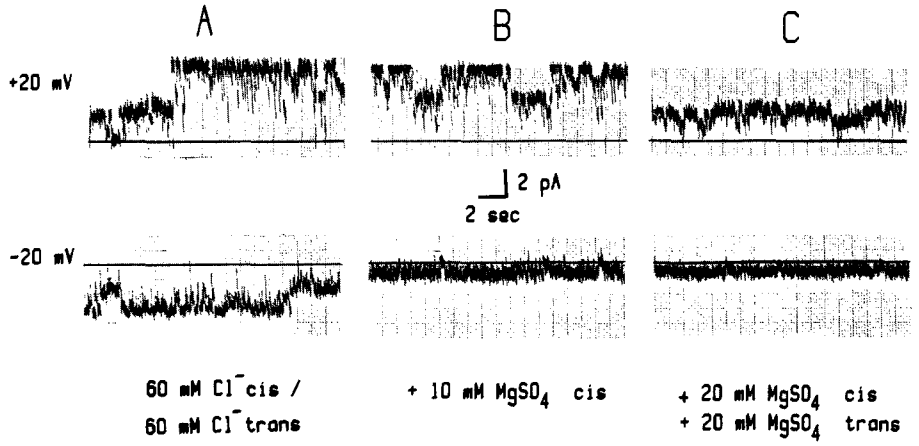


Fig. 7 A–C. Effects of $MgSO_4$ on a multi Cl^- channel recording in symmetric 60 m*M* Cl^-. **A** Chart recordings obtained in control conditions at +20 and −20 mV. **B** Same conditions as in **A**, but after addition of 10 m*M* $MgSO_4$ to the *cis* chamber. Cl^- ion transport is blocked from *cis* to *trans* as attested by the reduction of the current amplitude at −20 mV but not from *trans* to *cis*. No change of unit current amplitude was observed at +20 mV. **C** With 20 m*M* $MgSO_4$ present in both chambers. The unit current amplitude is decreased for both applied voltages, although the *cis* sulfate ion blocking effects were more pronounced than those from the *trans* side. The *solid line* represents the zero current level

DIDS, at submillimolar concentrations, efficiently and irreversibly inhibited Cl^- channel activity (not shown). The inhibitory effect of the stilbene derivatives SITS and DIDS on SR Cl^- permeability is well documented (Kasai and Kometani 1979) and has been used to eliminate Cl^- conductance in single channel recordings (Suarez-Isla et al. 1986).

Changes in pH from 8 (HEPES-*Tris* buffer) to 6.8 (PIPES-*Tris* buffer) failed to affect channel behavior but favored the rate of fusion of the SR vesicles. Other compounds such as ATP (Smith et al. 1985), taurine (Huxtable and Bressler 1973), and local anesthetics that have been shown to modulate the ionic permeability of intracellular membranes were without effect. Neither ATP (1 to 4 m*M*), taurine (up to 10 m*M*), lidocaine (up to 40 μM) or the calmodulin antagonist W7 (5 to 150 μM) showed a significant effect on Cl^- channel activity (data not shown).

Discussion

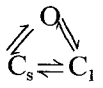
In this study we have described the basic functional properties of an anion selective channel of skeletal muscle SR vesicles. Whereas the SR Cl^- permeability has been investigated previously using isotopic flux measurements and light scattering techniques (Kometani and Kasai 1978), its properties at the single channel level have not been the subject of a detailed study. We observed single Cl^- channels which enabled us to test factors suspected of being involved in the regulation of this channel.

Chloride channels can be classified into three groups according to their conductance properties. The first one is made up of low conducting (>30 pS in 100 mM Cl^-) ligand-gated Cl^- channels (Hamill et al. 1983; Bormann et al. 1987). The second one has a conducting behavior (50–80 pS in 100 m*M* Cl^-) similar to that observed for the SR Cl^- channel in the present study. This group includes surface membrane Cl^- channels present in urinary bladder (Hanrahan et al. 1985), human tracheal epithelial (Welsh 1986) and cultured neurones (Franciolini and Petris 1988). Blatz and Magleby (1985, 1986) have reported the presence of two anion selective channels (45 and 61 pS in 100 m*M* Cl^-) in the surface membrane of cultured rat skeletal muscle. The kinetic properties, voltage dependence of activation and pH sensitivity of the two rat surface membrane Cl^- channels were however different from those observed for the rabbit SR Cl^- channel. A third group of Cl^- channels exhibited a high conductance (350–450 pS in 100 *M* Cl^-). These channels were observed in the outer mitochondrial surface membrane (Colombini 1979), in epithelial cells (Nelson et al. 1984), Schwann cells (Gray et al. 1984), macrophages (Schwarze and Kolb 1984) as well as in the surface membrane of skeletal (Blatz and Magleby 1985) and cardiac muscle (Coulombe and Duclohier 1984).

In the present study multiple subconducting states were observed in addition to a full open state with a maximum conductance of −154 pS (Fig. 3). The presence of subconducting states has been also observed for other Cl^- channels (Tank et al. 1982; Hamill et al. 1983; Schwarze and Kolb 1984; Gray et al. 1984; Miller and White 1984; Nelson et al. 1984) suggesting that the existence of substates is a general characteristic of Cl^- channels. They have been referred to as the mean amplitudes of less frequently occurring elementary current steps (Bormann et al. 1987). The reversal potentials of the SR Cl^- channel subconducting states, determined at different Cl^- concentrations were similar to the reversal potentials of the main conducting

state. Consequently, these lower amplitude events displayed a good Cl^- selectivity. Their overall contribution to SR Cl^- conductance is considered to be minimal due to their relative infrequent occurrence as compared to the full open state.

Within the limited time resolution (2 ms) of our bilayer measurements we were able to obtain evidence for the apparent existence of one open state and two closed states of the rabbit SR Cl^- channel. Accordingly, the kinetic behavior of the channel may be described by the following general scheme:



Where O represents the fully open state, and C_s and C_l the short and long closed states, respectively. At present it is unknown however whether all six transitions can occur. For the trout SR Cl^- channel a more complex general kinetic scheme has to be postulated in that our data analysis suggests the presence of a third closed state.

A low permeability of the organic anion $CH_3SO_3^-$ and inorganic anion SO_4^{2-} has been documented in ionic flux and light scattering measurements with SR vesicles (Kasai and Kometani 1979). Our experiments showed a decrease in the unitary Cl^- current amplitude by these large anions (Figs. 6 and 7). Two plausible causes for the decrease in current are a competitive blockage of the Cl^- channel due to the larger size of the $CH_3SO_3^-$ and SO_4^{2+} anions and/or a stronger interaction with an anionic binding site of the channel. Phosphate anions at concentrations lower than 5 m*M* did not affect the basic channel behavior in our experimental conditions. Higher phosphate concentrations (up to 20 m*M*) decreased the current amplitude without a significant effect on the open probability.

The effects of several physiological parameters possibly involved in the regulation or modulation of SR Cl^- channel activity in muscle were also studied. We failed to detect any significant modification of channel behavior when pH was changed from 8 to 6.8. Similarly, large variations in divalent cation concentrations (Ca^{2+} or Mg^{2+}) as well as the absence or the presence of ATP were without effect.

The sarcoplasmic reticulum membrane is permeable to various biologically relevant ions. At least three and possibly four separate passive permeation systems have been identified: (*1*) a Ca^{2+} release channel, (*2*) a K^+, Na^+ channel, (*3*) an anion channel, and (*4*) a H^+ (OH^-) permeable pathway which may or may not be synonymous with the anion channel (Meissner 1983). Recent studies have indicated that Ca^{2+} release channel activity is specifically associated with the junctional feet structures which span the gap between SR and T-tubule membranes (Inui et al. 1987; Lai et al. 1988). The Ca^{2+} release channel is believed to play a vital role in muscle function by releasing the calcium ions necessary for initiation of muscle contraction. In contrast to the Ca^{2+} release channel, the monovalent ion pathways are thought to be randomly distributed over the SR membrane (Meissner 1983). The physiological function of the monovalent ion channels is less clear, although it has been suggested that they may permit rapid movement of K^+, Na^+, H^+ and Cl^- across the SR membrane to counter electrogenic Ca^{2+} fluxes during rapid Ca^{2+} release and uptake (Meissner 1983; Garcia and Miller 1984).

In Table 1 are compared the single channel and ion permeability characteristics of the SR membrane of rabbit skeletal muscle. Although calculation of ion flux rates required various assumptions (see legend of Table 1) and is valid only in the case of independent ionic movements, it is nevertheless useful in providing an estimate of the major SR membrane ion conductances. Our data indicates that the Cl^- selective pathway is, in principle, capable of compensating electrogenic Ca^{2+} fluxes during Ca^{2+} release although, due to the low intracellular Cl^- concentration, it is however less efficient than the monovalent cation channel.

Table 1. Comparison of single channel and ion flux characteristics for SR terminal cisternae-derived vesicles of rabbit skeletal muscle

	Ion	γ	P_o	τ (for one channel)	N of channels/ vesicle	Ion flux rate
	[mM]	[pS]		[ms]		[ions/s/cm^2]
Ca^{2+} release channel	5	28	0.95	35	3	1.4×10^{15}
K^+ channel	100	130	0.60	59	2	9.5×10^{15}
Cl^- channel	5	5	0.85	54	4	1.0×10^{15}

Taus and ion flux rates for terminal cisternae-derived SR vesicles were calculated from literature data (Ca^{2+} release channel, Smith et al. 1986b; K^+ channel, Meissner 1983; Cl^- channel, present study) and using the expression $\tau = 4\pi z^2 F^2 c r^3 / 3\,RTN\gamma P_o$ (Garcia and Miller 1984) where c is the ion concentration, r is the radius, N is the number of channels per vesicle, γ is the estimated single channel conductance at c, and P_o is the probability of finding the channel open. Average vesicle radius of terminal cisternae derived vesicles was assumed to be 1.4×10^{-5} cm (Smith et al. 1986b). Z, F, R and T have the usual meanings

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Note added in proof

Tanifuji et al. (1987) J Membr Biol 99:103–111 have recently published data on a sarcoplasmic reticulum anion channel of larger conductance, with similar properties.

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